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(54) Improvements relating to antigens.

(57) A new glycoprotein 5T4 has been identified in human trophoblast. The antigen and fragments thereof and, more particularly, antibodies that recognise the antigen or fragments thereof are of value in relation to cancer diagnosis and treatment, particularly for the routine screening of cervical smears.

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fragments thereof as immunogen injected into small or large animals from whose blood the polyclonal antibodies are recovered by conventional methods. Monoclonal antibodies can be prepared utilising the 5T4 glycoprotein of the invention or fragments thereof or the 42 KDa core or fragments thereof as immunogen in a host animal, immortalising antibody producing cells of the host animal and recovering monoclonal antibody from the immortalised cells.

As an alternative to the use of the 5T4 glycoprotein of the invention, or its 42 KDa core or fragments thereof as immunogen in the raising of antibodies, one can also use a natural product including the 5T4 glycoprotein of the invention, isolatable from trophoblast cells. This material is known as syncytiotrophoblast glycoproteins, (StMPM), which can be isolated from human placenta by known methods. The 5T4 glycoprotein of the invention can be isolated from the StMPM by either antibody affinity chromatography or a combination of other chromatographic methods.

One particular monoclonal antibody that we have isolated and tested is one prepared by hybridoma techniques using StMPM wheat germ agglutin (WGA) glycoprotein as immunogen and which has become known as 5T4.

The antigens (5T4 glycoprotein, fragments thereof, the 42 KDa core and fragments thereof) of this invention and antibodies (that recognise antigens of this invention) are useful as diagnostic tools and in the production of vaccines. The purified 5T4 antigen for example allows the production of a family of related antibodies which recognise different epitopes of 5T4 antigen. Specifically, these antibodies are of interest:

i) in the development of contragestional vaccines since the antigen is expressed very early on in pregnancy;

ii) in foetal typing by the detection of foetal cells in the mother's bloodstream;

iii) as an early warning signal in situations of danger or damage to the foetus e.g. pre-eclampsia;

iv) in tumour screening and diagnosis in vitro and/or in vivo - in this respect it may offer significant advantage over antibodies to PLAP since the antigen is not found in pregnancy serum;

v) in routine monitoring of the female population with respect to premalignant conditions known as cervical intraepithelial neoplasia CIN 1, 2 and 3 detected in cervical biopsies. There is a correlation between the localisation and intensity of 5T4 reactivity in the dysplastic epithelium in CIN 2 and 3 preneoplastic lesions. The labelling intensity corresponds to the severity of the dysplasia with invasive carcinomas of the cervix strongly labelled.

Accordingly, the present invention includes compositions comprising the antigen or antibody of the invention together with a carrier or diluent. The exact nature of the carrier or diluent will depend upon the ultimate application of the antigen or antibody and, in the case where the antigen is to be used as a vaccine (or antibody as a passive vaccine) the carrier will be a parenterally acceptable liquid carrier. On the other hand, when the antigen or antibody is to be used for diagnostic purposes, the carrier may be liquid or solid and solid carriers for the antibody also represent a particularly important aspect of the present invention where the antibody is to be used as a means of purifying the naturally-occurring antigen by techniques of affinity chromatography.

The antigens and antibodies, immobilised or not, may be linked with radioisotopes or other revealing labels for localisation and/or therapy or conjugated with anti-tumour reagents for therapy. The antigen and antibody can be derivatised for use in different forms of assay for antigen concentration.

Specifically, the present invention includes a diagnostic test kit containing, as a solid component, an immobilised antigen or antibody of the invention and more specifically can contain, depending upon the specific type of assay to be used, an antigen and an antibody of the invention, one of which bears a revealing label. The antigen of the invention can be used in methods of in vitro or in vivo diagnosis targeting antibody while the antibody of the invention may be similarly used to target antigen. Such methods are of particular use in the diagnosis of various types of cancer, particularly for mass screening of cervical smears.

5T4 antigen has a relatively limited tissue distribution. It appears to be a pan-trophoblast marker which is expressed by all types of trophoblast examined as early as 9 weeks of development. It is specific for this tissue type within the placenta except for the amniotic epithelium which is also antigen positive. On the basis of immunoperoxidase staining of frozen sections from normal tissue, 5T4 antigen is also expressed by certain epithelial cell types. It should be noted that several 'trophoblast-characteristic' antigens, such as PLAP, are in fact found in normal tissues at trace concentrations (McLaughlin, 1986). Using a solid phase immunoassay to quantitate the expression of 5T4 relative to normal tissue, 5T4 antigen was found in placental plasma membrane in at least a 1000-fold higher concentration than that found in other normal tissues tested. However, this level of sensitivity would not necessarily detect expression in minor sub-populations of cells within a given tissue.

Several antibodies have exhibited a similar pattern of reactivity with normal epithelial tissues, for

Generation of Monoclonal Antibody

A male BALB/c mouse was immunised by six intra-peritoneal injections of WGA-purified StMPM glycoproteins (100-200µg/injection). Spleen cells were fused with NS1 murine myeloma cells (Kohler and Milstein, 1975), and the cells plated out in 24-well Linbro plates at $7x10^5$ cells/well. After two weeks, wells were assayed for StMPM reactive antibody by immunodotting. Positive clones were picked directly and further subcloned by limiting dilution. The antibody subclass was determined by double radial diffusion using a monoclonal isotype typing kit (Serotec, Bicester, U.K.). Antibody 5T4 was obtained by this technique.

Cell culture

Details of the cell lines described are found in table 3. Standard tissue culture media, alpha Dulbecco's modified Eagles medium (DMEM), DMEM or RPMI supplemented with antibiotics and 10-20% foetal calf serum (Gibco) were used.

Radioactive labelling of membranes and cells

Near confluent cell cultures of AV-3 cells were radiolabeled for 15-18 hours with ^{3}H-glucosamine (20 µCi/ml) (Amersham International) in RPMI containing 10% dialysed FCS. Metabolically labeled cells were collected and immunoprecipitated as follows : cells were removed from tissue culture flasks by incubation in 0.1M EGTA-PBS, washed in PBS (Dulbeccos-A) and then solubilized for 30 minutes at 4° C in 0.5% (v/v) NP40 in tris-buffered saline (TBS, 0.15M NaCl, 25mM Tris, pH 8.0) containing 0.1mM PMSF. Non-solubilized cellular components were removed by centrifugation at 14,000g and the amount of radioactivity incorporated into protein was determined following precipitation with 10% trichloroacetic acid.

Cell surface labelling by the lactoperoxidase-^{125}I method together with the techniques of immunoprecipitation and SDS-PAGE were carried out as previously described; high molecular weight standards (Sigma), red blood cell membrane proteins or ^{14}C-methylated protein mixtures (Amersham International) were used as marker proteins (Thompson et al., 1984; Stern et al., 1984; 1986). Tritiated sodium borohydride labelling of cell surface glycoproteins was carried out as described by Axelsson et al. (1978). Autoradiography and fluorography were as described in Thompson et al. (1984) using pre-flashed Fuji X-ray film.

Immunoperoxidase and immunofluorescence labelling

Immunoperoxidase staining of frozen tissue sections was carried out by the method of Bulmer and Sunderland (1983). Tissues were obtained as soon as possible post mortem, always within 12 hours, and processed immediately. Indirect immunofluorescence with cell suspensions was as described previously (Thompson et al., 1984). A monoclonal antibody we have isolated, directed against a widely expressed human antigen (mAb 1D2),was used as positive control.

Radiobinding assay of cell surface antigen expression

Cells were harvested with either EGTA-PBS or EGTA/trypsin, washed and resuspended in Earle's buffered saline solution (EBSS) with 0.5% bovine serum albumin and 0.1% sodium azide at $2x10^6$ cells/ml. The suspensions were plated out at 50µl (10^5 cells)/well in microtitre plates. 50µl mAb/well were added and incubated at room temperature for one hour. The cells were washed and $5x10^5$ CPM of ^{125}I-labelled $(Fab')_2$ fragments of sheep anti-murine immunoglobulin (Amersham International) added. Following incubation for one hour at room temperature, the cells were washed, harvested, and bound radioactivity determined on a gamma-counter. Assays were carried out in quadruplicate. Results are expressed as a ratio of specifically bound radioactive CPM relative to CPM with negative control antibodies. In some experiments 10^7 cells were incubated with 1ml of fixative (Buffered 10% formalin, Bouins' fixative, 0.25% gluteraldehyde, absolute ethanol or PBS control) for 30 minutes at room temperature and washed in EBSS. After incubation in 0.5% BSA in EBSS for 30 minutes, the cells were then processed as described above.

D-glucosamine in the same buffer. This fraction was loaded onto a mAb 5T4-sepharose affinity column (2 mg/ml ligand, 1 ml column). The IgG1 5T4 mAb was purified by high salt protein A affinity chromatography (loading buffer 1.5 M glycine, 3 M NaCl, pH 8.9. Elution buffer 100 mM citrate, pH 6.0) and bound to CNBr-activated Sepharose (Pharmacia). The mAb 5T4 affinity column was washed with 5 column volumes of 1% NP40/TBS and 5 column volumes of TBS. The bound 5T4 glycoprotein was eluted with 8 M urea. Fractions were assessed by immunodot (Stern et al, 1986), protein assay (Lowry, Rosebrough, Farr and Randall, 1951) and SDS-PAGE.

The WGA and 5T4 affinity chromatographic steps give a 10,000 fold purification with approximately 70% yield. Minor contaminants visible in silver stained SDS-PAGE are present at least 100-fold lower protein concentration than 5T4 antigen. Further fractionation by either Superose 12 gel filtration or hydrophobic interaction reverse phase chromatography yields 5T4 molecules devoid of contaminants.

RESULTS

The monoclonal antibody 5T4 is a murine IgG1. All work detailed in this study was carried out using subclone 5T4.B8. The preliminary screen by immunodot showed that the antigen recognised was none of the following major proteins associated with the trophoblast; IgG, transferrin, PLAP, HPL, albumin, calmodulin nor was it detectable in serum.

Tissue distribution

5T4 antigen expression in first trimester and full term placentae was investigated using indirect immunoperoxidase staining of frozen sections. Figure 1 illustrates antigen expression in term villous placenta as assessed by immunohistology of frozen sections. Villous trophoblast is strongly labelled by mAb 5T4, whereas the stroma is negative. There is specific labelling of the amniotic epithelium and extravillous cytotrophoblast of the chorion laeve but not of the amniotic mesenchyme or maternal decidua (Figure 1 c,d). Appropriate positive and negative controls are also shown; mAb 1D2 labels all parts of villi (fig 1a), mAb H316 labels trophoblast but is not specific for this tissue type (fig 1b; Stern et al., 1986); negative controls are unlabelled (Figs 1e, f). Extravillous cytotrophoblast in the placental bed is also labelled by mAb 5T4; no other element of the term placenta is 5T4 antigen-positive. Similar analysis of first trimester villous tissue has shown antigen expression by both syncytiotrophoblast and cytotrophoblast (data not shown). The earliest stage examined for 5T4 expression is in a chorionic villous biopsy at 9 weeks gestation which is positive by indirect immunofluorescence (with Dr.Bruce Smith, Jefferson, Philadelphia). This level of analysis suggests that 5T4 antigenic molecules are expressed by representatives of all subpopulations of trophoblastic cells.

5T4 was unreactive with the following non-pregnant tissues examined in immunohistology; spleen, heart, brain, liver, lung, bronchus, skeletal muscle, testis or ovary. Glomeruli in the kidney, villi of the small intestine, bladder epithelium, basal layer of the epidermis, endometrial glands of non-pregnant uterus and endocervical glands showed some specific labelling with mAb 5T4. Some small vessels in various tissues appeared to be weakly stained. Table I summarises 5T4 reactivity assayed by immunohistology of frozen tissue sections.

To further examine 5T4 expression, a semi-quantitative assay of 5T4 antigen on isolated membranes of some of the above tissues were assessed using solubilised proteins in an immunodot assay. 5T4 was still reactive with full term placental plasma membrane protein at an antigen concentration of 50ng/dot. In contrast to the widely distributed antigen recognised by mAb 1D2, 5T4 was not specifically reactive with any other tissue tested (ovary, testis, kidney, brain, liver, liver and muscle) at all antigen concentrations used (up to 50µg/dot). From this it was concluded that these normal non-gestational tissues express 5T4 antigen at approximately 1000-fold lower concentration than full-term placenta on a weight of crude membrane protein basis. This relative level of expression is comparable with PLAP as measured using mAb H317 (Table II).

Expression by cell lines

5T4 antigen expression by cell lines of normal and neoplastic derivation was assessed by indirect

amniotic epithelium. Normal mouse serum shows no labelling.

Figure 2

Immunoprecipitation of 5T4 molecules from StMPM. Autoradiography of SDS-PAGE analysis of 5T4 immunoprecipitates of NP-40 solubilised 125I-lactoperoxidase labelled StMPM (lane 1) and following digestion with N-glycanase (lane 2). 8% gel.

Figure 3

Fluorography of reduced SDS-PAGE of 5T4 immunoprecipitates from StMPM labelled with NaB^3H_4 following treatment with either periodate (PI) or galactose oxidase and neuraminidase (GO-N). 10% gel. T is total radiolabelled glycoprotein following periodate treatment.

Figure 4

Gel filtration of 5T4 antigenic molecules. Solubilised StMPM protein fractionated over S200 Sephacryl in the presence of detergent. Fractionated 5T4 antigenicity assessed in ELISA.

TABLE I

REACTIVITY OF MONOCLONAL ANTIBODY 5T4 WITH NORMAL HUMAN TISSUE AS ASSESSED BY IMMUNOHISTOLOGY OF FROZEN SECTION.

Tissue	Result
Placenta	+ + + Villous trophoblast and amnion
Brain	-
Ovary	-
Testis	-
Skeletal muscle	-
Heart	-
Lung	-
Bronchus	-
Liver	-
Spleen	-
Kidney	+ Glomeruli
Bladder	+ Epithelium
Small intestine	+ Villous epithelium
Uterus	+ Endometrial glands
Cervix	+ Endocervical glands
Skin	+ Basal epidermis

TABLE III

REACTIVITY OF MAB 5T4 WITH NORMAL CELLS AND TRANSFORMED CELL LINES IN CELL-SURFACE IMMUNOFLUORESCENCE AND RADIOBINDING ASSAY.

Cell	Origin	Type	RESULT Fluore-scence	Binding index	Reference
AV-3	Amion	Epithelial	+	3.1	McLaughlin et al., 1982.
WISH	Amion	Epithelial	nt	(3.4)	Gift of P.McLaughlin, Liverpool
MRC-5	Fibroblasts	Embryonic	+	3.8 t	Jacobs et al., 1970.
Flow 7000	Fibroblasts	Embryonic	nt	(2.9)	Gift of P.McLaughlin, Liverpool
I407	Intestine	Embryonic	+	nt	Gift of A.Smith, Clatterbridge
PBL	Peripheral blood	Leucocytes	−	nt	(1)
UC729/6	B-cell	Myeloma	−	nt	Gift of A.Smith, Liverpool.
HMI	"	Myeloma	−	nt	Gift of A.Smith, Liverpool
RAJI	"	Lymphoblastoid	−	1.2	Pulvertaft, 1964.
BSM	"	Lymphoblastoid	−	1.2	Gift of Dr. C.Graham, Oxford.
Daudi	"	Burkitt's lymphoma	−	1.2	Klein et al., 1967.
BZ7	"	EBV-lymphoblastoid	−	1.1	Gift of Prof.C.Hart, Liverpool.
Molt-4	T-cell	Leukaemia	−	nt	Minowada et al., 1972.
K562	"	Erythroleukaemia	−	1.2	Andersson et al., 1979.
GCDM/15	Brain	Glioma	+	5.2 t	Gift of Dr. T.Alderson, London.
Hep-2	Larynx	Carcinoma	+	(5.0)	Moore et al., 1955.
HN2	Larynx	"	+	(1.5)t	Easty et al., 1981
HN4	Larynx	"	+	3.0 t	"
HN1	Tongue	"	+	2.9 t	"
HN5	Tongue	"	+	3.1 t	"
IPT	Bronchus	"	−	1.2 t	Kumar et al., 1983.
IPTV2	Bronchus	"	−	1.3 t	Walker et al., 1984
N417	Small lung	"	−	1.2	Gift of Dr.T.Alderson, London
6CT	Cervix	"	+	2.2	Daniels et al., 1984
E1Co	Breast	"	nt	1.7	Gift of P.McLaughlin, Liverpool
EJ	Bladder	"	+	nt	O'Toole et al., 1983.
A431	Vulva	"	+	4.2 t	Fabricant et al., 1977.
HT29	Colon	"	+	3.4 t	Gift of A.Smith, Liverpool.
Mavi	Colon	"	−	nt	Gift of A.Smith, Liverpool.
Chang	Liver	"	nt	(4.1)	Gift of P.McLaughlin, Liverpool
Tera-1	Testis	Teratocarcinoma	+	(2.6)	Fogh and Trempe, 1975.
Tera-2	Testis	"	+	4.2	Thompson et al., 1984.
2102Ep	Testis	"	+	(3.5)	Andrews et al., 1984.
PA-1	Ovary	"	+	(4.1)	Zauthan et al., 1980.
Bebo	Chorion	Choriocarcinoma	+	(5.2)	Patillo and Gey, 1968.
Jar	Chorion	"	+	(4.9)	Patillo et al., 1971.
SK-NEP	Kidney	Wilm's tumour	−	(1.2)	Fogh and Trempe, 1975.
Gos.1.8.1	Kidney	"	−	(1.4)	Gift of Dr. C.Graham, Oxford.
GMBOB	Kidney	"	+	(5.1)t	Gift of Dr. T.Alderson, London.

Cells harvested with EGTA alone or EGTA-trypsin (t). Cells incubated with mAb 5T4 followed by fluorescein-conjugated sheep anti-murine Ig (immunofluorescence) or ^{125}I rabbit anti-mouse immunoglobulin (Binding assay). Results expressed as positive immunofluorescence or binding index relative to negative control. Standard deviation of four replicates was less than 10%; variation between 2-4 experiments was generally less than 10%. Figures in brackets represent results from a single experiment. (1) PBL isolated from peripheral blood by centrifugation over Ficoll-hypaque. nt = not tested.

unreactive with MAb 5T4.

Table II summarises the distribution of MAb 5T4 in neoplastic tissues. Many of the malignant epithelial tumours displayed positive reactions in the neoplastic cells. Of note, were carcinomas of breast (5/5), lung (5/5), stomach (6/7) and pancreas including one of the ampulla of Vater (4/4). Also positive, albeit in only a limited number of cases available, were carcinomas of endometrium and cervix.

The majority of colonic adenocarcinomas were negative, positivity in 3/12 was confined to only a few tumour cells and was weak.

Cystadenocarcinomas of the ovary produced variable reactions. In three of the four positive cases the majority of tumour cells were positive, and in the other the majority were negative.

In the testes all classical seminomas were negative; only a seminoma with syncytiotrophoblast-like giant cells and admixed with embryonal carcinoma being positive. All anaplastic germ cell tumours of the testis showed variable positive reactions. Where syncytiotrophoblast was present this was strongly positive. Generally embryonal carcinoma and yolk sac structures were only feintly positive. This ranged from the majority of tumour cells being positive (1 case) to a minority (1 case). Undifferentiated mesenchyme may also be positive.

The cystic epithelium of mature teratomas often displayed a focal weak to moderate reaction.

Syncytiotrophoblast of choriocarcinomas and a complete hydatidiform mole was strongly positive. Much of the trophoblast of placental site tumours showed moderate or strong labelling on both cell membranes and within the cytoplasm. Single examples of fibrosarcoma and leiomyosarcoma showed that whilst most tumour cells were negative, there were focal and weak reactions in a few cells. Malignant melanomas (2) and malignant lymphomas (3) were negative.

The stroma of some tumours showed weak and focal reactions. This was also noted in the endothelium lining mainly small blood vessels in many tissues and tumours.

The cellular location of binding with mAb5T4 in tumours may be either membranous or cytoplasmic or a combination of both. Heavy membrane-bound location is a particular feature of syncytiotrophoblast. Cytoplasmic reactivity was predominant in pancreatic carcinomas. In gastric and breast carcinomas both types of pattern were present. MAb 5T4 was unreactive with fixed and paraffin wax embedded tissue sections of villous trophoblast of term placentae.

Comment

MAb 5T4 gives reactions in trophoblast which are similar to other antitrophoblast antibodies. However our detailed immunohistochemical analysis tends to suggest that the antigen recognised is distinct from HCG,HPL,PLAP and those which react with mAb 18A/C4 and 18B/A5 (Loke, University of Cambridge). Some of the differentiating immunohistochemical features are summarised below.

Unlike antibodies to HCG, 5T4 will react with some non-HCG producing tumours and gives intense reactions with syncytiotrophoblast of term placenta. Antibodies to both HCG and HPL are unreactive with the basal layer of stratified squamous epithelium and normal or non-neoplastic endocervical glands.

Seminomas, usually positive with mAbs against the Nagao isozyme of PLAP, (egH17/E2) were almost all negative using 5T4. (The one case that showed some positivity was a seminoma containing syncytiotrophoblast giant cells, admixed with embryonal carcinoma. MAb 5T4 in contrast to mAbs reactive with the Regan isoenzyme of PLAP was usually negative with bronchiolar epithelium.

The failure of mAb 5T4 to react in fixed and routinely processed paraffin sections is a characteristic of some other reported antibodies directed against membrane-associated antigens, notably anti-PLAP, 18A/C4 and 17.1A antibodies.

Tissue/Organ	Morphology	Number positive	Intensity staining	Distribution/Comments
Lung	Non-neoplastic lung taken from metastases	1/5	?+++	Difficult to assess whether these are alveolar lining cells, type II pneumocytes or degenerate tumour since from a case of choriocarcinoma otherwise lung parenchyma in other cases is negative
Lymph node	Non-specific reactive changes	1/1	+/to+	Clusters of cells in sinusoids faintly positive, probably endothelial cells and histiocytes.
Oesophagus	Non-neoplastic	2/2	+	Basal layer of stratified squamous epithelium
Ovary	Non-neoplastic including corpus luteum, corpora albicantra and stroma	0/4		Almost all negative apart from faint focal +/- of stromal cells. Surface epithelium and follicles not seen.
Prostate gland	Hyperplasia	1/1	+/-to +	Focal of glandular epithelium, most negative
Pancreas	Normal or non-neoplastic	3/3	+	Focal, faint staining of small collecting duct cuboidal epithelium and mucus secreting epithelium. Most acinar cells negative but focal +/-
Seminal vesicles	Normal	1/1	+/ to +	Focal of epithelium. Most negative

TABLE VI

Results of Immunohistochemical Distribution of Monoclonal Antibody 5T4 in Neoplastic Tissues

Tissue/Organ	Morphology	Number positive	Intensity of staining	Distribution/Comments
Ampulla of Vater	Invasive adenocarcinoma	1/1	+++	Focal staining of tumour acini Membrane and cytoplasmic
Bladder	Poorly differentiated carcinoma with squamous differentiation	1/1	++	Focal positivity of some tumour cells membrane and cytoplasmic. Most tumour cells negative
Brain	Glioblastoma multiforme	0/1		
Breast	Invasive adenocarcinoma	5/5	+ to ++	Usually membrane and cytoplasmic staining of tumour cells. Occasional +/- 'wispy' staining of stroma
Cervix	Invasive squamous carcinoma	1/1	+++	Cytoplasmic & membrane in most tumour cells, endocervical glands show ++ to +++ and + to ++ stromal cells.
Colon	Invasive adenocarcinoma	3/12	+	Focal, of few tumour cells only. Weak +/- to + of stroma and non-neoplastic large bowel mucosal glands
Colon	Tubulovillous adenoma	1/1	++	Mainly membrane (mucosal surface) with some cytoplasm.
Endometrium	Invasive adenocarcinoma	1/1	+ to +++	Small groups of cells +++ membrane, focal ++ staining of undifferentiated and multinucleate cells.

Tissue/Organ	Morphology	Number positive	Intensity of staining	Distribution/Comments
Ovary	Brenner Tumour (in mucinous cystadenoma)	1/1	+	Clusters of Brenner tumour only positive, cytoplasm
Ovary	Granuloma cell tumour	0/1		
	Cystadenoma	0/3		Weak +/- focal of mucin
	Cystadenocarcinoma various sites	4/7	++ to +++	Positive tumours, both membrane and cytoplasm. In 3 cases most tumour cells positive and appox. 50 of tumour cells positive in 1 case. Negative tumours - serous papillary (xl) mucinous (xl), poorly differentiated (xl)
	Teratoma, solid	1/1	+/- to +++	Basal layer of squamous epithelium +/-, respiratory epithelium +, focal in mucin secreting cells. Mesenchyme and chondrocytes +/- to + Acini ++ to +++
Pancreas	Invasive adeno-carcinoma	3/3	+/-to++	Focal, mainly cytoplasm with little membrane. Many tumour cells negative Stroma +/- to ++
Skin	Basal cell carcinoma	0/1		
	Malignant melanoma	0/2		In one case, a very few cells faintly positive, otherwise all tumour cells negative.

Tissue/Organ	Morphology	Number positive	Intensity of staining	Distribution/Comments
Teratoma	Anaplastic germ cell tumours, including three metastases, two MTI, one of which is metastatic	7/7	+/- to +++	Trophoblast +++, M. Embryonal carcinoma/yolk sac tumour + Undifferentiated tumour, possibly neurol, ++. Note, these tumours are variable in their reactions and in some many tumour cells are negative
Thyroid	Adenocarcinoma metastatic to thyroid, (unknown primary)	0/1		
Trophoblast	Choriocarcinoma (x 2 in uterus x 1 in lung x 2 in brain)	5/5	+ to +++	Syncytiotrophoblast ++ to +++, M. Cytotrophoblast + to ++ in one case
	Placental site Trophoblast tumour	2/2	++ to +++	Most tumour cells, mainly membrane, some cytoplasm
	Hydatidiform mole	1/1	+ to +++	Syncytiotrophoblast +++ membrane staining; faint + staining of cyto-trophoblast. Stroma of chorionic vil negative

* M M M T = Malignant, Mixed Mullerian Tumour

** including serous cystadenocarcinoma x 2
mucinous cystadenocarcinoma x 1
metastatic ovarian carcinoma in lymph node x 1

read independently by two observers - some material was poorly preserved and not included in the summary. Placental villous sections were included in each experiment to ensure that the procedure was working optimally. The degree of labelling was assessed as anything above that shown in the negative control, eliminating the possibility of false positives becoming included into the study. A subjective estimation of the intensity of the labelling was also made. Experiments were repeated at least once on greater than 50% of the specimens.

RESULTS

5T4 Expression in cervical biopsy frozen sections

Table VII summarises the extent of 5T4 labelling from the basal epithelium to the surface in 66 cone or punch biopsies from the ectocervix. The data can be grouped in several categories. The sections of "normal" ectocervix, squamous metaplasia and HPV infection without evident dysplasia exhibited an overlapping phenotype in intensity and range of distribution of 5T4 antigen. 9/17 showed labelling confined to the basal cells of the epithelium; 6 showed faint labelling throughout the epithelial layers and only 2 demonstrated significant labelling to level C3. There was labelling associated with the stromal elements to the same degree as the basal layer: columnar epithelium and glands when present were labelled. These results are in the range of those described for cervical tissue in a previous immunohistological study of 5T4 expression in normal and neoplastic tissues.

The above arbitrary grouping shows no obvious differences from the specimens in the CIN 1 category. The latter is characterised by the appearance of atypical nuclei located in the lower third of the epithelium. Where the morphology was preserved in the frozen sections, it was frequently noted that the 5T4 labelling was located in the parabasal layers corresponding to the area of dysplasia.

From the data on Table VII, it is apparent that there is a progression through CIN 2 and CIN 3 to a more extensive pattern and intensity of labelling with 5T4 monoclonal antibody. The staining is of higher intensity than in that detected generally in the non-dysplastic or CIN 1 specimens. All the CIN specimens frequently exhibited stromal labelling with an intensity to the basal layers. Where the morphology of dysplastic cells could be assessed, it was evident that from the CIN 2 (HPV) and CIN 3 categories that the specific 5T4 labelling associated with the abnormal cells. 14/15 CIN 3 showed labelling from the basal layer to just below the surface epithelium; 9/15 exhibited labelling along the surface. 5/5 examples of squamous cell carcinoma showed positive intense labelling of the malignant cells and surrounding stroma. The final group of miscellaneous conditions includes hyperplasia, chronic inflammation, cervicitus, acanthotic epithelium and radiation induced atypia. These specimens were selected on the basis of their conventional pathology and exhibited a range of labelling. The inflammatory infiltration response did not increase 5T4 expression per se; the single example of acanthotic epithelium was clearly labelled as were 2/3 of the hyperplastic epithelia. This arbitrary grouping shows some tendency to higher levels of 5T4 expression in the centre layers but appears different from the CIN 2 and CIN 3 groupings.

A new approach using a tumour marker specific for cervical cancer may revolutionise current methods for screening, by offering the potential for the tumour specific Ag to be detected in serum and mucous samples and solubilised biopsies.

Observing the 5T4 antigenic distribution over a wide range of malignant and premalignant conditions in cervical cancer, a consistent pattern of staining for specific pathological disorders was evident. Normal cervical epithelium, one of the 'specialised' epithelia, showed faint reactivity localised to the reserve cells only. CIN, being the progressive transformation from normal to the malignant state, demonstrated an increased pattern of epithelial labelling corresponding to the severity of the dysplasia. Labelling of the stroma, glands and vessel endothelium also increased as the malignant potential progressed, with no evidence of reactivity with inflammatory cells. Invasive carcinoma showed strong staining of the malignant cells and the intervening stroma. Anaplastic tumours showed patchy variable labelling which may reflect the transformed cells' ability to modulate their morphology and antigenic characteristics. The labelling of the atypical cells is consistent with the theory of the malignant lesion commencing from the basal layers and spreading to the surface.

The quantitative assessment of 5T4 antigenicity in cervical smear material using radio or other immunoassay with 5T4 monoclonal antibodies may be used as a means of assessing the degree of dysplastic cells in a specimen. This procedure can be highly efficient in mass screening and assigning further investigative procedures.

Pathology	Specimen Number	Epithelial layer C1	C2	C3	C4	C5
CIN 3	38	+	++	++	++	++
	39	+	+	++	++	++
	40	-	+	+	++	++
	41	-	+	+	++	++
	42	+	+	+	+	++
	43	-	+	+	++	++
	44	++	++	++	++	++
	45	-	+	+	+	+
	46	+	+	++	++	++
CIN 3 with HPV	49	+	+	+	++	++
	50	+	+	++	++	++
	51	-+	-	-	+	++
	52	+	+	+	+	++
Invasive Carcinoma	53	Epithelial layers not present tumour in stroma				++
Hyperplasia	58	+	+	+	+	++
	59	-	-	-+	-+	-+
	60	+	+	+	+	+
Chronic Inflammation	61	-	-	-	-	++
	62	+	-+	-+	-+	+
Cervicitis & glandular atypia	63	-+	-+	-+	-+	-+
		-	-	+	+	++;
Acanthotic Epithelium	65	+	+	+	+	+
Radiation induced atypia	66	-	-	-	-	-

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Claims

1. 5T4 antigen which is a glycoprotein characterised by the following properties:

a. Molecular weight of 72 KDa on SDS-polyacrylamide gel electrophoresis (PAGE) under reduced conditions; 69 KDa under non-reducing conditions.

b. Monomeric structure in the plasma membrane as judged by gel filtration and two-dimensional SDS-PAGE-IEF (iso-electric focusing). Approximate isoelectric point = 6.9.

c. Removal of N-linked sugars with N-glycanase reveals a 42KDa core structure.

25. A method according to claim 24 for the diagnosis of cervical cancer.

26. An antigen according to claim 1, 2, 3, or 16 or an antibody according to claim 10, 11 or 14 for use in a method of treatment by surgery or therapy practised on the human or animal body or in a method of diagnosis practised on the human or animal body.

27. A method of treatment to introduce antibody into a host in need of such treatment which comprises parenterally administering to the host an effective amount of an antigen according to any one of claims 1, 2, 3 or 16 or of an antibody according to any one of claims 10, 11 or 14.

Fig.2.

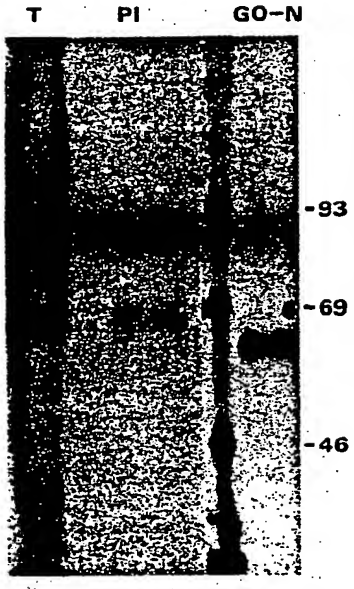


Fig.3.

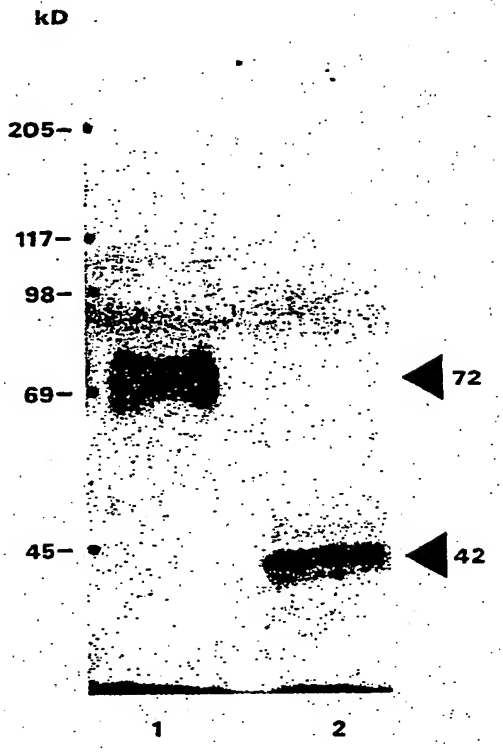
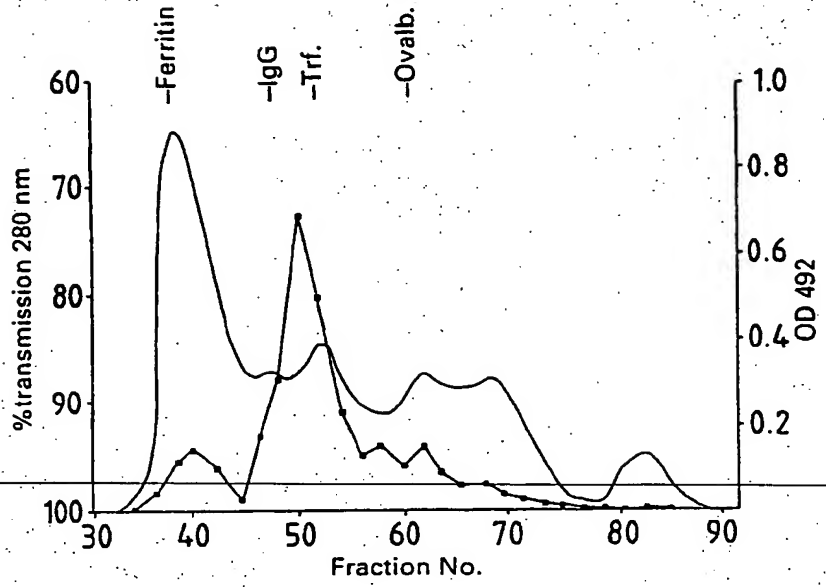


Fig.4.



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PARTIAL EUROPEAN SEARCH REPORT

Application number
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-2-

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
A,D	AMERIC. JOURNAL REPROD. IMMUNOL. vol. 1, 1981, pages 246 -254, Alan R. Liss, Inc., US; P.M. JOHNSON et al.: "Human trophoblast-specific surface antigens identified using monoclonal antibodies" --		
A,D	INT. J. CANCER, vol. 35, no. 4, 1985, pages 469-475, Alan. R. Liss, Inc., US; W.J. RETTIG et al.:"Cell surface antigens of human trophoblast and choriocarcinoma defined by monoclonal antibodies" ------		TECHNICAL FIELDS SEARCHED (Int Cl.4)

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